

- Sub C1
- BI
- (ii) adjusting the pH to an acid pH with a strong acid;
 - (iii) separating the mixture from step (ii) into two layers;
 - (iv) removing the upper layer and adjusting its pH to approximate neutrality;
 - (v) adding to the product from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;
 - (vi) adjusting the pH of the product from step (v) to the alkaline side with a weakly alkaline aqueous solution; and (vii) separating out the cell wall C-polysaccharide antigen containing not more than 10% protein;
- c) coupling to a chromatographic column through a spacer molecule the cell wall C-polysaccharide antigen obtained in step b;
- d) passing antibodies to *Streptococcus pneumoniae* over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and
- e) conducting an assay upon a liquid sample suspected of containing *Streptococcus pneumoniae*, which assay comprises the step of detecting the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*, if present, by contact of the liquid sample with a detection agent which essentially comprises purified antigen-specific antibodies from step (d) hereof, which may in part be conjugated to a tag, whereby that a detectable physical or chemical change is effected as a result of contact of the detection agent with the sample.

34 The method of claim 32 in which the spacer molecule of step (c) is a protein molecule.

35 The method of claim 33 wherein the sample of step (e) is a natural fluid of mammalian origin.

36 The method of claim 35 wherein the liquid sample of step (e) is human urine.

Sub C) 37 The method of claim 36 in which the fluid sample is taken from a patient exhibiting clinical signs of pneumonia.

38 The method of claim 37 in which the sample is taken from a patient exhibiting clinical signs of otitis media.

BI 39 The method of claim 35 wherein the liquid sample of step (e) is human spinal fluid.

40 The method of claim 39 wherein the sample is obtained from a patient suspected of having developed meningitis.

~~41 The method of claim 33 in which step (e) is an immunoassay process.~~

42 The method of claim 40 in which step (e) is an immunochromatographic ("ITC") process.

43 The method of claim 33 in which step (e) is conducted by

Sub C) a) contacting a liquid sample suspected of containing *Streptococcus pneumoniae* or its cell wall C-polysaccharide antigen, with an ICT device comprising a housing containing a strip of bibulous material, which strip has

- (i) a first zone in which has been movably embedded a conjugate of a labelling agent with purified antigen-specific antibodies obtained in step (d) of claim 33, said labelling agent being selected from among those known to display a visible color change upon the formation of a labelled antibody: antigen: fixed antibody reaction product and
- (ii) a second zone having fixedly bound thereto unconjugated purified antigen-specific antibodies from step (d) of claim 33, which zone is equipped with a window in the housing for viewing color changes,
- b) allowing said liquid sample to flow laterally along said test strip to said first zone where it picks up the movably embedded conjugate of labelling agent and antibodies;
- c) allowing said liquid sample and said conjugate of antigen-specific antibodies to flow laterally together along said test strip to said second zone, and
- d) within not more than 20 minutes after first contacting the liquid sample with the test strip, observing whether a line of color, indicating the presence in the sample of *Streptococcus pneumoniae* or its cell wall C-polysaccharide antigen, has formed.

44 The method of claim 43 wherein the sample is a natural fluid of mammalian origin.

45 The method of claim 45 wherein the sample is human urine.

46 The method of claim 45 wherein the sample is taken from a patient exhibiting overt clinical signs of pneumonia or another respiratory tract illness known to be often caused by *Streptococcus pneumoniae*.

47 The method of claim 44 wherein the liquid sample is human spinal fluid.

48 The method of claim 45 wherein the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

49 The method of claim 45 wherein the liquid sample is taken from a patient suspected of having developed meningitis.

50 An ICT device for the detection of *Streptococcus pneumoniae* bacteria which comprises a housing containing a strip of bibulous material having

- Sub C2
- BI
- a) a first zone in which has been movably embedded a conjugate of a labeling agent and purified antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, and
 - b) a second zone downstream of said first zone having immovably bound thereto a portion of purified antibodies specific to the same cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, which zone is equipped with a window in the housing for viewing color changes, which antibodies are further characterized in that their antigen specificity has been attained by passing antibodies to *Streptococcus pneumoniae* over a chromatographic affinity column to which is coupled a spacer molecule conjugated to a purified cell wall C-polysaccharide antigen obtained from a culture of *Streptococcus pneumoniae* bacteria according to the following method:

- (i) harvesting cells from the said culture in the form of a wet cell pellet;
- (ii) suspending the wet cell pellet in an alkaline solution and mixing;
- (iii) adjusting the pH of the resultant mixture to an acid pH with a strong acid;
- (iv) separating the acidified product from step (iii) into two layers;
- (v) removing the upper layer and adjusting its pH to approximate neutrality;
- (vi) adding to the product from step (v) a broad spectrum protease enzyme and digesting to destroy residual proteins;
- (vii) adjusting the pH of the product from step (vi) to the alkaline side with a weakly alkaline side with a weakly alkaline aqueous solution; and
- (viii) separating out the cell wall C-polysaccharide of *Streptococcus pneumoniae* having no more than 10% protein.

51 The ICT device of claim 50 wherein the labelling agent is finely divided metallic gold.

52 A method for detecting *Streptococcus pneumoniae* in a liquid sample which comprises

- a) contacting said sample with the strip of bibulous material of the ICT device of claim 49;
- b) allowing the liquid sample to flow laterally to the first zone of said test strip where it picks up the movably embedded conjugate of labelling agent and purified antigen-specific antibodies;
- c) allowing the liquid sample and entrained conjugate to flow laterally along said test to the second zone thereof; and